Original Article

Expression of stathmin in oral squamous cell carcinoma and its correlation with tumour proliferation

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Abstract

Background: Stathmin is a member of microtubule-associated protein. Inhibition of Stathmin expression can interfere with tumour progression and also alter the sensitivity of tumour cells to microtubule-targeting agents. Thus, it could be a potential therapeutic target for planning new treatment strategies.

Objective: To study expression of Stathmin in different histological grades of oral squamous cell carcinoma (OSCC) and its correlation with Ki67 index.

Materials and Methods: This study was an observational retrospective and prospective study conducted during a period of two and half years from January 2015 to June 2017 at ESI-PGIMSR Maniktala, Kolkata where 52 cases of OSCC were studied. Haematoxylin and eosin sections were reviewed and representative paraffin blocks were selected. Immunostains were performed using antibody clones for Stathmin and Ki67. For Stathmin scoring, Segersten scoring system was applied. Statistical analysis was done by Graph Pad Prism using Krusher Wallis Test and one-way ANOVA test. Spearman's coefficient was used to establish corelation between Ki 67 and Stathmin overexpression.

Results: In this study, it is found that strong Stathmin expression score (4–9) was detected mostly (82.35%) in moderately differentiated (MD) OSCC and poorly differentiated (PD) OSCC (100%), whereas in contrast, 60% of well-differentiated OSCC showed negative-to-weak Stathmin score (1–3). Mean Ki67-labelling index for well-differentiated carcinoma was 32.37%, for moderately differentiated carcinoma was 60.89, and poorly differentiated carcinoma was 86.15%, which demonstrated increased tumour cell proliferation with progression of histological grades of OSCC.

Conclusion: Stathmin expression was higher in MD OSCC to PD OSCC compared to well-differentiated carcinoma and its overexpression was significantly correlated with Ki67 index. Thus, Stathmin is overexpressed in higher grades and is correlated with high proliferation of tumour with a potential role as therapeutic target.

Keywords: Histological grade, Ki67 labelling index, oral squamous cell carcinoma, stathmin expression

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is not only the most common carcinoma of oral cavity but also the most common carcinoma of head and neck.[1] Even with currently available treatment modalities, the survival rate has not been improved substantially as patients are often diagnosed at late stages.

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This highlights the need to discover suitable biomarker for early diagnosis of the disease and to understand the disease pathogenesis as a first step towards improving survival.

Stathmin1 is an oncoprotein and it is one of the members of microtubule-associated protein family. It is basically a ubiquitous cytosolic phosphoprotein and key regulator of cell division because of its depolymerisation of microtubules in a phosphorylation-dependent manner. Assembly of mitotic spindle requires dephosphorylation and inactivation of Stathmin before cell enters into mitosis[2] The ability of Stathmin to remodel microtubules indicates its role in tumour migration and invasion. Microtubules are essential component of the cytoskeleton that play a critical role in cell division and cell survival. Hence, it has been a potential target for chemotherapeutic agent such as vinca alkaloids that engage mitotic spindle checkpoints arresting cell cycle progression and leading to apoptosis.[3] Overexpression of Stathmin decreases polymerization of microtubules and consequently decrease the sensitivity of vinca alkaloids. Studies have shown that Stathmin is overexpressed in many human cancers and bears significant relationship with grade and prognosis. In a meta-analysis done by Biaoxue *et al*.,[4] it was observed that overexpression of Stathmin promotes metastasis and growth of solid malignant tumours. Ju *et al*.[5] established that overexpression of Stathmin is associated with poor survival in head and neck cancers. This indicates that an effective therapeutic approach could be designed that combines Stathmin inhibition with microtubule-targeting agents. Furthermore, Koike *et al*.[6] observed upregulation of Stathmin in OSCC-derived cell lines compared to normal oral keratinocytes.

Ki67 antigen is a nuclear non-histone protein that is found in proliferating cells. Proliferation is a fundamental process that plays a major role in growth and maintenance of tissue homeostasis.[7] Studies have shown that expression of Ki67 increases with severity of dysplasia. Studies have also shown that cell proliferation in invasive tumours measured by ki67 highly correlated with histological grade in OSCC.[8] Additionally, Ki67 serves as marker of tumour aggressiveness because of its positive association with tumour proliferation and invasion.[9]

This study evaluates the differential expression status of Stathmin in different histological grades of OSCC and its correlation with Ki67-labelling index.

MATERIALS AND METHODS

This study is an observational, retrospective, and prospective study performed during a period of two and half years from January 2015 to June 2017 at ESI-PGIMSR Maniktala, Kolkata. The study population was histopathologically diagnosed cases of OSCC in the Department of Pathology, ESI-PGIMSR Manicktala. Patients who have received neoadjuvant chemotherapy were excluded from the study. After seeking permission from the Institutional Ethics Committee (IEC No- ESIPGI/MKT/IEC/9/2016) and informed consent from patients, data were collected. Clinical data, such as age, gender, symptoms, location, and extension of the tumour, nodal involvement were taken. Collection of patient profile data such as tobacco and alcohol habits were also obtained from medical records.

For histopathological study, specimens were fixed in 10% neutral-buffered formalin. Tissues were processed embedded in paraffin. Sections were taken and stained with haematoxylin and eosin (H and E). After histopathological diagnosis of squamous cell carcinoma, cases were graded as well-differentiated (WD), moderately differentiated (MD), and poorly differentiated (PD) carcinomas. A representative block for each case was selected for Immunohistochemistry (IHC). Four-micrometre sections were cut and mounted on poly-L-lysine-coated slides taking care to mount the section flat and wrinkle free. Slides were incubated for 30 min at 60°C. Slides were deparaffinised and then rehydrated in graded alcohol and then put to water. Antigen retrieval was done using TRIS EDTA buffer at pH 9 in a microwave in 3 cycles for about 20 min. After cooling down to room temperature, slides were washed in wash buffer (pH 7.4) and endogenous peroxidase blocking was done with 3% hydrogen peroxide for 10 min. IHC was done using primary antibody (Rabbit monoclonal antibody cloneSP49 for Stathmin and Rabbit monoclonal antibody clone: SP6 for Ki67) and slides were incubated for 60 min at room temperature in a moist chamber. Again, after wash with wash buffer, slides were incubated in the moist chamber with secondary antibody (horse radish peroxidase labelled). The reaction product was detected with 3,3- diaminobenzidine chromogen. Counter staining was done by haematoxylin. Sections were again dehydrated in graded alcohol and mounted with DPX. IHC slides were scored by three pathologists individually by scoring system suggested by Segersten *et al*.[18] that is depicted in Table 1. Final score was calculated as the product of intensity score and extent of staining, and final score was evaluated as score 1–3 weak and score 4–9 was considered as moderate to strong. Standard statistical methods (mean, standard deviation, Krusher Wallis test, one-way ANOVA test, and Spearman's coefficient) were applied for analysis of data using Graph Pad Prism version 8.4.2 (679).

RESULT

A total number of 52 patients were included in this study group who fulfilled the inclusion criteria. Out of 52 cases, 24 cases were small tissue biopsy samples. Clinicopathologic characteristics are depicted in Table 2. Age range of the study population was 31–77 years with mean age of 53.28 years. Majority of the patients were males, Male: Female ratio being 3.3:1. Tumours were located at tongue (12 cases, 23.07%), buccal mucosa (19 cases, 36.5%), cheek (07 cases, 13.4%), alveolus (07 cases, 13.4%) retromolar (05 cases, 9.61%) and lower lip (02 cases, 3.8%). H and E revealed 30/52 (57.69%) cases of WD OSCC, 17/52 (32.69%) of MD OSCC, and 05/52 (9.61%) of PD OSCC.

IHC done with Stathmin revealed 18 out of 52 (34.61%) cases having an overall Stathmin score ranging from 1 to 3 [Figure 1c]. Out of these, 15 (83.33%) cases were WD OSCC [Figure 1a]. Thirty-one out of 52 (59.61%) cases showed moderate to strong expression with their score ranging from 4 to 9 [Figure 2b]. Of all 30 cases of WD OSCC, 15 cases (50%) showed weak expression of Stathmin and 03 cases (10%) showed negative expression [Figure 1b]. Out of 17 cases of MD OSCC [Figure 2a], 3 (17.6%) cases showed weak Stathmin expression, whereas 14 cases (82.35%) showed moderate/strong expression. All five cases (100%) with PD OSCC [Figure 3a] showed strong Stathmin expression [Figure 3b] with an overall score ranging from 6 to 9 [Table 3].

Table 1: Segersten *et al*. scoring system

Intensity Score	Extent Of Staining
Weak - 1	<25% -1
Medium - 2	25-75%- 2
High-3	>75%- 3

Table 2: Clinicopathologic characteristics of patients

Charecteristic	Number Of Patients (*n*=52)
Age	
Mean	53.28
Range	31-77
Sex	
Male	40
Female	12
Type of specimen:	
Small biopsy	24
Others	28
Location of the tumour:	
Tongue	12
Buccal mucosa	19
cheek	07
Alveolus	07
Lower lip	O5
Retro molar growth	02
Grade of the tumour:	
Well differentiated	30
Moderately differentiated	17
Poorly differentiated	05

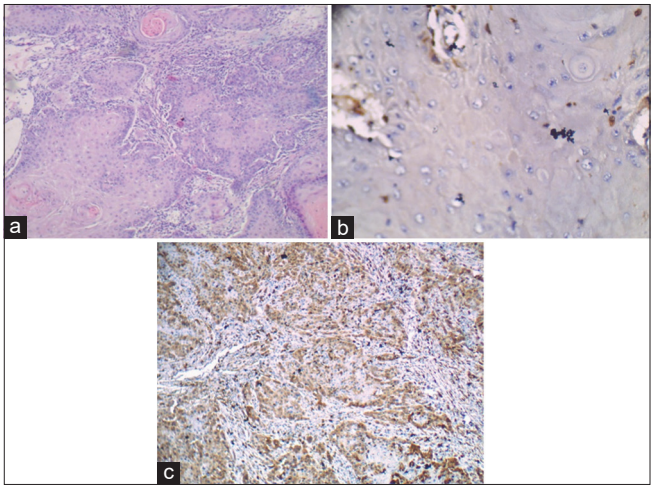


Figure 1: Photomicrograph of well-differentiated oral squamous cell carcinoma (a) H&E stain ,100 x, (b) Negative Stathmin expression Immunostain with Stathmin, 400x, score 0, and (c) Stathmin immunostain with weak positivity 100x, score 3

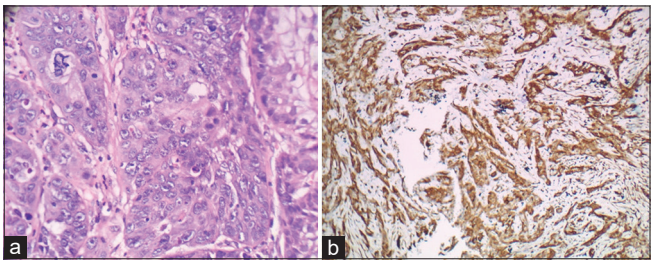


Figure 2: Photomicrograph of Moderately differentiated squamous cell carcinoma (a) H&E stain X400 and (b) Immunostain with Stathmin of same showing moderate staining ,100x

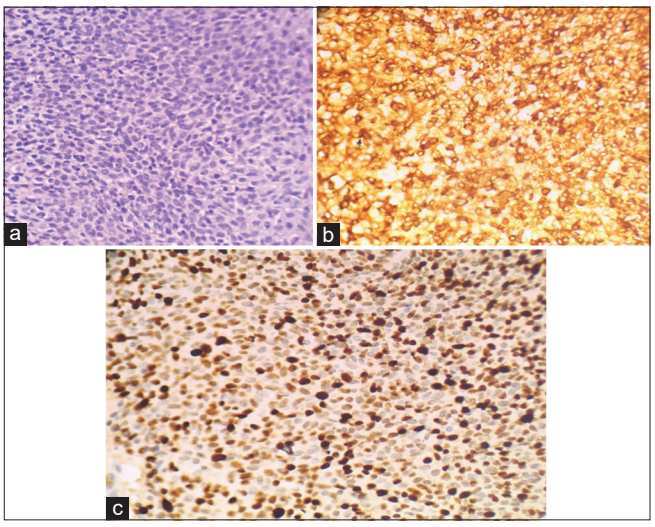


Figure 3: Photo micrograph of poorly differentiated squamous cell carcinoma (a) H&E stain X100, (b) Immunostain with Stathmin showing strong immune positivity 400x, stathmin score 9, and (c) Immunostain with Ki67

Mean value of Stathmin score for WD OSCC was compared with that of MD OSCC and PD OSCC as demonstrated in Graph 1. Mean score of WD OSCC was 2.88 ± 2.24 SD, against 4.82 ± 1.78 SD in MD OSCC and 8.4 ± 1.34 in PD OSCC [Table 4]. The data were analysed by Kruisher Wallis test for multiple comparisons using Graph Pad Prism version 8.4.2 (679). *P* value of less than 0.05% was considered significant. When Stathmin results were compared, it was found that strong Stathmin expression score (4–9) was detected mostly (82.35%) in MD OSCC and PD OSCC (100%), whereas in contrast, 60% of WD OSCC showed negative-to-weak Stathmin score (1–3).

A statistically significant difference was observed when Stathmin expression was compared between WD OSCC and MD OSCC (*P* value 0.0291) and between WD OSCC and PD OSCC (*P* value 0.0003). However, no statically significant difference was detected when Stathmin score was compared between MD OSCC and PD OSCC (*P* value 0.0931) shown in Table 4.

Mean Ki67-labelling index for WD OSCC was 32.37% with a standard deviation of ± 22.89, whereas mean Ki67-labelling index for MD OSCC was 60.89 with a SD of ± 17.34 and PD OSCC was 86.15% [Figure 3c] with a standard deviation of 5.78 [Table 5]. Statistical analysis done using one-way ANOVA Test demonstrated a significant difference of mean Ki67 expression between WD OSCC and MD OSCC (*P* value <0.0001), WD OSCC and PD OSCC (*P* value <0.0001), and a *P* value of 0.0478 between MD OSCC and PD OSCC. Hence, it was concluded that expression of Ki67 protein increased progressively according to grades of OSCC that reflects tumour proliferation with increasing grades.

Table 3: Stathmin positivity with respect to tumour differentiation

Grade Of The Tumour	Negative Expression Score 0	Weak Expression Score: (1-3)	Moderate/Strong Expression Score: (4-9)
Well differentiated (*n*=30)	03	15	12
Moderately differentiated (*n*=17)	00	03	14
Poorly differentiated (*n*=5)	00	00	05
Total (*n*=52)	03	18	31

Table 4: Mean intensity score of Stathmin in various histological grades of OSCC (*P* Value * not significant)

Histological Grade (Number of cases=52)	Stathmin Score (Mean±SD)	*P*
WD OSCC (*n*=30)	2.87±2.24	0.0291
MD OSCC (*n*=17)	4.82±1.28	0.0003
PD OSCC (05)	8.4±1.34	0.0931*

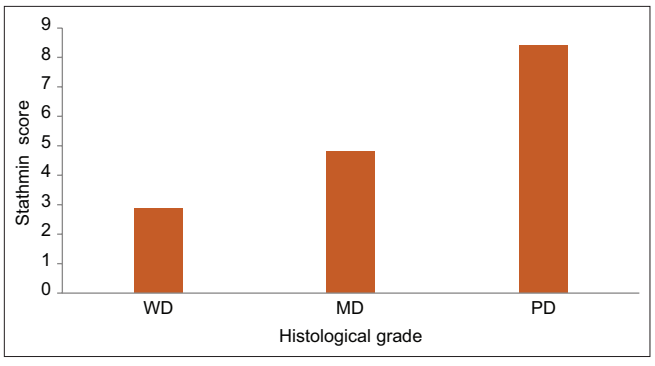
Table 5: Mean Ki67-labelling index with respect to tumour differentiation

Grade Of Tumour (*n*=52)	Number of cases	Mean Ki67 Index	SD	*P*
Well differentiated	30	32.37	27.89	<0.0001
Moderately differentiated	17	60.89	17.34	<0.0001
Poorly differentiated	05	86.15	5.78	0.0478

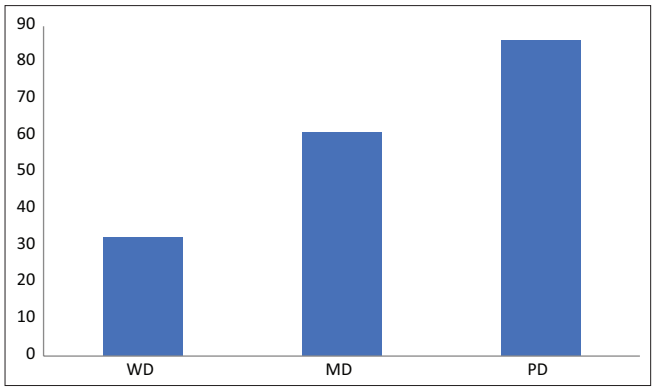
When Stathmin results were compared with histological grades and mean Ki67-labelling index, it was found that mean Ki67 protein expression increased with progression of grades of OSCC from WD OSCC to PD OSCC as depicted in Graph 2. Stathmin expression was found to be higher in MD OSCC and PD OSCC compared to WD OSCC. When the mean of Ki67 and Stathmin score was compared statistically by Spearman's correlation coefficient, strong Stathmin expression was observed to be positively corelated as demonstrated in Graph 3.

DISCUSSION

Koike *et al*.[6] showed upregulation of Stathmin in OSCC-derived cell lines. In this study, it was observed that there was statistically significant increased expression of Stathmin with increasing grades of OSCC. This study demonstrated a statistically significant increased expression of Stathmin from WD OSCC to MD OSCC and PD OSCC. Among the Intragroup comparison of different grades of OSCC, there was statistically significant increase from WD OSCC to MD OSCC and PD OSCC. However, no statistically significant increased expression of Stathmin from MD OSCC to PD OSCC was found. In a study by Vadla *et al*.[10], a statistically significant corelation was found between increased grades of OSCC and oral dysplasia and overexpression of Stathmin, which is in concordance with present study. Apart from OSCC expression of Stathmin, its expression has been studied on various tissues. Study by Liu *et al*.[11] established overexpression of Stathmin in oesophageal carcinoma and its significant corelation with histological grades. Baquero *et al*.[12] evaluated association



Graph 1: Stathmin expression with grades of OSCC

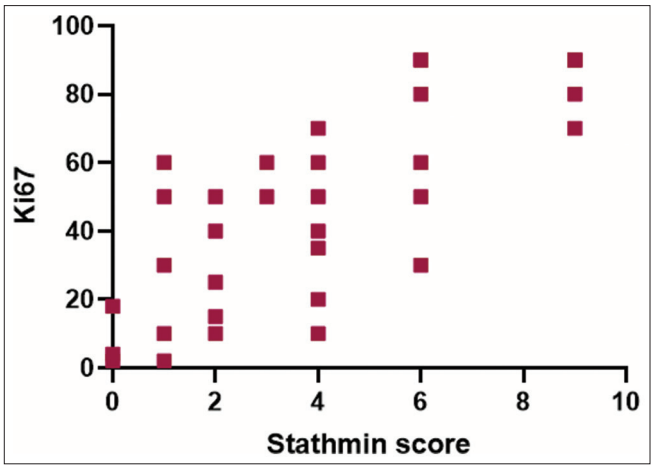


Graph 2: Correlation between grade of OSCC and Ki67 expression

of Stathmin expression in breast tissue with overall survival using Kaplan Meier analysis, patients with low Stathmin expression showed improved survival compared with those who had high expression.

Dwivedi *et al*.[7] demonstrated different expression patterns of Ki67 as a marker of proliferating cells in different degrees of epithelial dysplasia and OSCC and found that expression of Ki67 index in OSCC increased according to the histological grade of OSCC. Macluskey *et al*.[13] showed statistically significant correlation with mean Ki67 index in healthy tissue, dysplasia, and in carcinomas thereby suggesting that epithelial proliferation may continue to increase during the transition from dysplasia to carcinoma. Study done by Jing *et al*.[9] established that Ki67 protein overexpression was associated with progression of OSCC and also serves as an independent prognostic marker for OSCC patients. This study found that mean Ki67 was 32.37% in WD OSCC, 60.89% in MD OSCC, and 86.15% in PD OSCC that demonstrates increased tumour cell proliferation according to histological grades as measured by Ki67 protein expression. This is consistent with previous studies done by Dwivedi *et al*.[7] and Takkem *et al*.[14] Alhough the expression of Stathmin and Ki67 index have been extensively studied separately on various tissues in various parts of the world, none of the literature to the best of our knowledge have studied correlation of Stathmin and Ki67 on OSCC so far. In this study, it was found that strong Stathmin expression was noted in higher grades and showed significant association with tumour cell proliferation as measured with Ki67-labelling index.

Studies by Mistry and Atweh have shown that inhibition of Stathmin expression in malignant cell interferes with their orderly progression through the cell cycle.[15] In study done by Ju *et al*.,[5] it was found that OSCC patients with low Stathmin expression benefited from induction therapy with TPF (Docetaxel, Cisplatin, and 5 Flurouracil) compared to OSCC patients with high Stathmin expression. They concluded that Stathmin overexpression promoted



Graph 3: Correlation between Stathmin score and Ki67 expression

cell proliferation and decreased OSCC sensitivity to TPF-based induction chemotherapy. Liu *et al*.[11] studied expression of Stathmin in human gastric cancer showed that Stathmin might be a potential molecular marker and target for treatment of gastric cancer. Ma *et al*.[16] found that Stathmin is overexpressed in OSCC and it could be a potential antitumor therapeutic target in OSCC. In addition, Nishio *et al*.[17] showed that Stathmin-transfected lung cancer cell line has increased sensitivity to vinca alkaloids. These observations suggest that Stathmin inhibition when combined with pharmacologic agents that stabilise the mitotic spindle could serve as a potential therapeutic target.

CONCLUSION

Based on this study, it is concluded that Stathmin expression was significantly overexpressed in MD OSCC and PD OSCC compared to WD OSCC and its overexpression was significantly corelated with Ki67 index. Thus, Stathmin is overexpressed in higher grades and is correlated with high proliferation of tumour. However, for evaluation of prognostic and therapeutic implications of Stathmin, long follow up and larger study population is needed.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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